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An interesterified palm olein test meal decreases early-phase postprandial lipemia compared to palm olein: a randomized controlled trial.

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ABSTRACT

Palm oil that has been interesterified to produce a higher proportion of palmitic acid (16:0) in the *sn*-2 position reduces postprandial lipemia in young, normolipidemic men and women, but effects in older subjects with higher fasting triacylglycerol (TAG) concentrations are unknown. We tested the hypothesis that high-fat meals rich in interesterified palm olein (IPO) decrease lipemia and alter plasma lipoprotein fraction composition compared to native palm olein (NPO) in men aged 40-70 y with fasting TAG concentrations ≥ 1.2 mmol/L. Postprandial changes in plasma lipids following meals containing 75 g fat (NPO and IPO) were compared using a randomized, double-blind crossover design (n=11). Although there were no significant differences in plasma TAG concentrations between meals over the total 6 h postprandial measurement period, IPO resulted in a decreased plasma TAG response during the first 4 h of the postprandial period (iAUC 1.65 mmol/L.h, 95% CI 1.01-2.29) compared to NPO (iAUC 2.33 mmol/L.h, 95% CI 1.58-3.07); meal effect $P = 0.024$. Chylomicron fraction TAG concentrations at 4-6 h were slightly reduced following IPO compared to NPO (NPO – IPO mean difference 0.29 mmol/L (95% CI -0.01-0.59), $P=0.055$). There were no differences in IDL fraction TAG, cholesterol or apolipoprotein B48 concentrations following IPO compared with NPO. In conclusion, consuming a meal containing palm olein with a higher proportion of 16:0 in the *sn*-2 position decreases postprandial lipemia compared to native palm olein during the early phase of the postprandial period in men with higher than optimal fasting triacylglycerol concentrations.

KEY WORDS

Palm olein; Interesterification; Triacylglycerol; Postprandial lipemia;

25 ABBREVIATIONS

26 iAUC Incremental area under the curve

27 DVP Digital volume pulse

28 IPO Interesterified palm olein

29 NEFA Non-esterified fatty acids

30 NPO Native palm olein

31 TAG Triacylglycerol

32

33 INTRODUCTION

34 Following consumption of a fat-containing meal, circulating triacylglycerol (TAG)
35 concentrations are elevated for up to 8h. As reviewed recently, three recent
36 prospective studies have confirmed non-fasting TAG concentrations as a strong
37 predictor of risk of coronary heart disease, and to be more discriminatory than fasting
38 TAG concentrations [1-4]. Long chain saturated fatty acids such as palmitic acid
39 (16:0) result in pronounced postprandial lipemia, but the magnitude of the increase in
40 blood TAG concentrations may depend on the positional composition of the
41 triacylglycerol [5].

42 Interesterification is an industrial process to shuffle the position of fatty acids within
43 the TAG molecule without changing the fatty acid composition of the TAG and is
44 commonly used by the food industry to produce fats with desirable physical
45 characteristics. Interesterification of palm oil, which has 16:0 predominantly in the
46 outer positions of the TAG molecule (*sn*-1 and *sn*-3), increases the proportion of 16:0
47 in the *sn*-2 position and results in a fat with a higher melting point than native palm
48 olein (NPO), the latter being liquid at room temperature. The transition within the oils

and fats industry away from animal fats and *trans* fatty acids towards interesterified palm olein (IPO), for use as a solid vegetable fat in bakery products and fat spreads, has recently prompted a number of scientific investigations into the acute and chronic health effects of interesterified fats, particularly in relation to their atherogenic potential [5-10]. Animal studies have demonstrated that consumption of TAG containing palmitic acid in the *sn*-2 position promotes atherogenesis to a greater extent than TAG containing palmitic acid the *sn*-1 or *sn*-3 positions [11]. However chronic human studies have not found an effect of positional composition of palmitic acid on cholesterol concentrations [12, 13], and there have been no studies to date investigating longer term markers of atherogenesis.

With regard to postprandial lipemia, the evidence to date is fairly consistent in showing a decreased postprandial TAG response to IPO compared to NPO during the initial phase of the postprandial period. Specifically, Yli-Jokipii and colleagues reported decreased iAUC for plasma TAG concentrations after 55 g/m² body surface area IPO compared to NPO in 10 healthy premenopausal women with a mean age 27 y (SD 3) [14], Berry *et al.* reported decreased plasma TAG concentrations 1-2 h after 50 g IPO compared to NPO in 20 healthy young men with a mean age of 29 y (SD 10) [7], and Sanders *et al.* reported decreased plasma TAG concentrations 1-4 h after 50 g IPO compared to NPO (and high oleic sunflower oil) in 50 healthy young men and women with a mean aged of 24 y (SD 3) [10]. These three study populations were all young, with mean fasting TAG from 0.7 to 1.1 mmol/L (SD 0.4 to 0.5), and mean body mass indices of 21 to 23 kg/m² (SD 2-3). Sanders *et al.* concluded that fats that are solid at room temperature with a higher proportion of palmitic acid at the *sn*-2 position decrease postprandial lipemia compared with liquid oils, but that this requires replication in older subjects [10]. The relative postprandial

lipemic response to IPO in a study population considered to be more “at-risk” of developing cardiovascular disease has not yet been characterized. Furthermore, differences in postprandial lipemia that may arise following IPO compared to NPO may result in compositional differences in circulating lipid fractions which may differ in their atherogenic potential; for example, a slower rate of increase of TAG-rich lipoproteins in the early phase of postprandial lipemia may be associated with a preponderance of TAG-rich lipoproteins and their remnants in the later postprandial phase.

The present study set out to test the hypothesis that interesterified palm olein, with an increased proportion of palmitic acid in the *sn*-2 position, would decrease postprandial lipemia in middle-aged/older men with higher than optimal fasting plasma TAG concentrations, but would increase the concentration of TAG in the lipoprotein fraction rich in TAG-rich lipoprotein remnants (the “IDL” fraction) in the later postprandial period. The primary outcome measure was incremental area under the curve (iAUC) for plasma TAG, and secondary outcome measures included TAG and cholesterol content of lipid subclasses. Other outcome variables included blood pressure and vascular tone assessed by digital volume pulse (DVP). This is the first study to date to investigate compositional changes (in TAG, cholesterol and apo-B48 concentrations) in lipid subclasses following a single meal of IPO compared to NPO.

SUBJECTS AND METHODS

Subjects

Ethical approval for the study was obtained from a National Health Service research ethics committee (NRES Committee London – Dulwich reference 11/LO/0116), and written informed consent was given by participants. This trial was registered at

clinicaltrials.gov as NCT01710280. Advertisements and a circular email within King's College London were used for recruitment, which was initiated in May 2011 by research investigators. A participant information sheet was provided to volunteers who expressed interest. Participants, recruited from King's College London and the wider local community, attended the Metabolic Research Unit at the Diabetes & Nutritional Sciences Division, King's College London in a fasting state for a screening appointment which included the measurement of height, weight, waist circumference, % body fat (by bioelectrical impedance using the Tanita™ Body Composition Analyser), seated blood pressure, liver function tests, glucose, lipid profile and haematology. A small remuneration was given for participation in the postprandial study days. Since the target population was middle-aged/older men with higher than optimal fasting plasma TAG concentrations, inclusion criteria were: healthy males aged 40-70 y; non-smoking; and fasting plasma TAG ≥ 1.2 mmol/L. The initial aim was to recruit men with fasting plasma TAG ≥ 1.5 mmol/L but this was adjusted to ≥ 1.2 mmol/L due to difficulties recruiting sufficient numbers of volunteers. Exclusion criteria were as follows: reported medical history of cardiovascular disease, cancer, liver, kidney or bowel disease; fasting glucose ≥ 6.1 mmol/L or uncontrolled type 2 diabetes; presence of gastrointestinal disorder or use of drug which is likely to alter gastrointestinal motility or nutrient absorption; history of substance abuse or alcoholism; current self-reported weekly alcohol intake exceeding 28 units; allergy or intolerance to any component of the test meals; unwilling to restrict consumption of any source of fish oil for the duration of the study; weight change of >3 kg in preceding 2 months; body mass index <20 and >35 kg/m²; fasting blood cholesterol >7.8 mmol/L; and current use of lipid lowering medication. Sample size calculations were based on 80% power at $P = 0.05$ to detect a 1.4

mmol/L.h difference in the iAUC for plasma triacylglycerol concentrations, which gave a sample size of 11.

Study design

A randomized, double-blind, crossover design was used to compare 2 test meals that did not differ in appearance, aroma or taste. Each test meal consisted of a muffin and a milkshake and provided 4.38 MJ (1047 kcal), 14 g protein, 93 g carbohydrate, and 75 g test fat. The 2 test fats were as follows: native palm olein (NPO, iodine value 56, Intercontinental Speciality Fats, Selangor, Malaysia) and interesterified palm olein (IPO, iodine value 56, Wilmar PGEO Edible Oils, Johor, Malaysia). Both palm olein (PO) fats had a similar fatty acid composition (shown in **Table 1**), but the IPO has 45.9 mol% of palmitic acid in the *sn*-2 position compared to 9.8 mol% in NPO [5]. The test fats were baked into muffins, labelled with a code A or B by an independent research technician, and stored frozen until consumed. The allocation of treatment was blinded from both the investigators and the study participants.

Subjects were randomly allocated to receive either meal A or B for their first visit by a single research investigator using a computer randomisation program. The participants consumed each of the 2 test meals in random order, and each test meal protocol was separated by at least 1 week. On the day preceding each test meal, participants were told not to participate in strenuous exercise and to avoid alcohol and foods high in fat. They were provided with a standardized low-fat meal (containing <10 g fat) as their evening meal, which they were required to consume before 2200 h and then to avoid eating or drinking anything except for water. Participants attended the Metabolic Research Unit between 0800 and 1000 h the

next day. A cannula was inserted into the forearm antecubital vein, and blood was collected for baseline analysis. Following a 10 min supine rest, blood pressure and digital volume pulse measurements were taken in a supine position. The test meal was consumed within 10 min. Further venous blood samples were collected hourly until 6 h after the test meal and blood pressure/vascular measurements were made 2, 4 and 6 h after the test meal. Participants had access to water to sip as required over the 6 h period. After the blood sample at t+3 h, they consumed a light, fat-free lunch (1.7 MJ consisting of 190 g fat-free yoghurt (<0.1 g fat) and a 120 g banana) as in previous studies [6, 7, 10, 15].

Methods

Blood samples for total plasma fatty acid composition, plasma TAG fraction fatty acid composition, plasma NEFA fraction fatty acid composition, plasma TAG, cholesterol, and NEFA concentrations were collected into EDTA tubes and centrifuged at 1500 x g for 15 min at 4 °C. Two millilitres plasma from blood samples taken at 4, 5 and 6 h time points was mixed with 0.77 g potassium bromide and 50 mg sucrose, then added to Ultraclear ultracentrifuge tubes (Beckman Coulter UK Ltd, catalogue number 344060), and over-layered with solutions of sodium chloride, potassium bromide and 100 mg/L EDTA sequentially at densities of 1.225 g/ml (2 ml) and 1.100 g/ml (4 ml), ending with distilled water containing 100 mg/L EDTA (4 ml), according to the method of Terpstra and colleagues [16, 17]. Ultracentrifugation using a SW40 Ti swinging bucket rotor (Beckman) in a Beckman-Coulter Optima L-90K ultracentrifuge at 160,070 x g for 42 min at 20 °C with brake to 500 rpm was used to isolate the chylomicron-rich fraction (aspirating the top 1 ml layer), then the ultracentrifuge tubes were refilled with distilled water and ultracentrifuged at 230,501 x g for 23 h at 22 °C to obtain the “IDL” layer which contains VLDL, IDL and

chylomicron remnants. For ease of nomenclature these lipoprotein fractions will be referred to as the chylomicron fraction (density <1.006 kg/L) and IDL fraction respectively (density 1.006-1.019 kg/L), although it is recognised that both fractions will also contain VLDL. Further aliquots of plasma obtained from blood collected hourly 0-6 h were stored frozen at -80 °C until analysed. Enzymatic assays were used to determine concentrations of NEFA (WAKO NEFA-HR; WAKO Diagnostics), cholesterol, and TAG on an ILAB-650 analyser (Instrumentation Laboratories). The plasma TAG and NEFA fractions were separated by TLC and analyzed by GLC to determine fatty acid composition [18, 19]. Apolipoprotein B48 concentrations in IDL fractions were measured using a human apo B48 ELISA kit (Shibayagi Co. Ltd., Shibukawa, Japan). Total apolipoprotein B concentrations were measured using a polyethylene glycol (PEG) enhanced immunoturbidimetric assay (Siemens Healthcare Diagnostics Ltd, Surrey, UK), however results for this analysis are not available due to a technical error that occurred during analysis. The intra-assay coefficients of variation were: NEFA, 6.3%; TAG, 3.4%; cholesterol, 1.0%; and apolipoprotein B-48, 7.1%.

Blood pressure was measured according to British Hypertension Society guidelines using an automated upper arm blood pressure monitor, the Omron 705IT (Omron Healthcare Europe B.V.). The digital volume pulse was obtained by photoplethysmography (PulseTrace, Micro Medical Ltd., Kent, UK) and used to calculate stiffness index (DVP-SI, m/s) and reflection index (DVP-RI, %).

Statistical analyses

An incremental AUC (iAUC) was calculated for plasma TAG, NEFA and cholesterol concentrations and paired t-test carried out to compare meal differences. Repeated

measures ANOVA was performed (meal and time as within-subject factors) on change from baseline data for total plasma analytes, or raw data for lipoprotein fraction data which was only collected at 4-6 h. A P value <0.05 was considered statistically significant.

RESULTS

Twenty-five volunteers who met the initial eligibility criteria following a telephone questionnaire attended screening sessions at the Metabolic Research Unit, Diabetes & Nutritional Sciences Division, King's College London, of whom 14 did not meet inclusion criteria (**Figure 1**). The screening characteristics of the 11 participants who completed the study (June-December 2011) are shown in **Table 2**.

The postprandial changes in plasma TAG and the iAUC after the test meals are shown in **Figure 2**. There were no significant differences between meals although both the treatment effect for plasma TAG changes from baseline (1-6 h) ($P = 0.067$) and the iAUC 0-6 h ($P = 0.09$) showed a trend towards a smaller increase in postprandial plasma TAG concentrations following IPO compared to NPO. This was more marked in the early phase of postprandial lipemia and *post hoc* analysis of the iAUC from 1-4 h was conducted to determine the early phase postprandial TAG response. There was a significant difference between meals, with a decrease in Δ plasma TAG 1-4 h ($P = 0.020$) and in the iAUC 0-4 h ($P = 0.024$) observed following IPO compared to NPO. There were no differences between meals for changes or iAUC in plasma cholesterol or NEFA concentrations.

The TAG concentration of the chylomicron fraction (4-6 h) was decreased following IPO compared to NPO but this did not quite reach significance ($P = 0.055$) (**Table 3**). However, a paired T-test at the point of peak lipemia (4 h) showed that chylomicron

TAG was significantly lower after IPO compared to NPO (mean difference NPO-IPO 0.463 mmol/L, 95% CI 0.032-0.894 mmol/L, $P = 0.038$). There were no differences in the TAG concentration of the IDL fraction, nor the cholesterol concentrations of the chylomicron and IDL fractions. Furthermore, no differences in IDL fraction apolipoprotein B48 concentrations were observed, although there was a large amount of inter-individual variability (table 3). The fatty acid composition of the plasma TAG fraction was close to the test meal fat composition at 4-5 h, the point of peak lipemia (**Table 4**). The proportion of plasma NEFA and TAG fractions that was 16:0 increased ($P < 0.0005$ and $P < 0.005$ respectively) and the TAG fraction 18:1 *n*-9 proportion increased ($P < 0.005$) following the meals, but the proportion of the NEFA fraction that was 18:1 *n*-9 initially decreased at 1-2 h ($P < 0.005$). Comparison between the test meals revealed that 16:0, 18:0, 18:1 *n*-9 and 18:2 *n*-6 profiles following NPO and IPO were similar.

There were no significant meal effects or meal x time interactions for blood pressure or digital volume pulse (Supplementary Figure 1).

DISCUSSION

Evidence points to an independent role for non-fasting (postprandial) triglyceride concentrations as a risk factor for cardiovascular disease [20], and calculated non-fasting remnant cholesterol concentrations have been associated with risk of coronary heart disease [21]. This study aimed to investigate whether the interesterification of palm olein resulted in adverse effects on postprandial lipemia in healthy men aged 40-70 y with higher than optimal fasting TAG concentrations. As previously reported in younger men and women [7, 10, 14], a blunted increase in plasma TAG during the early, pre-peak phase of postprandial lipemia was observed

following IPO compared to NPO. We decided to investigate an older, male population who had high-normal levels of fasting TAG and were likely to have a more pronounced postprandial lipemia [22], to confirm that the same response occurs in those who do not have overt cardiovascular disease or type 2 diabetes, but may have raised metabolic risk factors.

Importantly, these results confirm that interesterification influences postprandial lipemic responses even in individuals with higher fasting TAG levels and a more pronounced lipemic response. The reduction in incremental AUC for plasma TAG during the early phase was in the order of 30%, which is considered clinically relevant. However, due to the lack of clinical prevention trials using standardized postprandial TAG measurements the implications of this size of reduction on risk of cardiovascular events is unknown. Furthermore the relative importance of the magnitude versus the duration of postprandial lipemia on cardiovascular risk is unknown. Quintiles of random measurements of non-fasting TAG concentrations that differed by a mean of ~0.3-0.5 mmol/L were associated with significantly increasing hazard ratios for coronary heart disease mortality in men from the Norwegian Counties Study, suggesting that postprandial differences of a similar size to that observed here could be clinically significant if these fats form a major component of a habitual high-fat diet [4]. The postprandial TAG response was approximately 1.5 mmol/l higher at the 4 h peak concentration compared with our previous study [10], but it is also important to note that the total amount of fat was greater in the current study: 75 g compared to 50 g used previously [10]. A meal containing 75 g fat was administered because an objective of the study was to analyse the lipid composition of the chylomicron and IDL fractions, and by inducing a maximal increase in

268 postprandial TAG-rich lipoprotein (TRL), the detection of any meal-induced
269 differences would be optimized.

270 Prolonged lipemia is expected to result in compositional changes in lipid fractions
271 due to the reciprocal exchange of TAG and cholesterol via CETP between
272 chylomicron and HDL and LDL particles. It was hypothesised that although IPO
273 might elicit a beneficial reduction in the early postprandial phase response (0-4 h) a
274 subsequently prolonged postprandial lipemic response (> 4 h) would result in greater
275 lipoprotein remodelling by CETP and therefore a higher remnant TAG concentration
276 and greater TRL cholesterol content. The cholesterol content of TAG-rich
277 lipoproteins is thought to be an important causal factor in the development of
278 atherosclerosis [23]. The concentration of TAG in the chylomicron fraction was
279 moderately reduced 4-6 h following IPO compared to NPO, reflecting the reduction in
280 total TAG concentration in plasma. This did not result in differences in the
281 TAG:cholesterol ratio in the chylomicron and IDL fractions from 4-6 h. Furthermore,
282 the IDL fraction apolipoprotein B-48 concentrations at 4-6 h did not appear to be
283 affected by interesterification of palm olein, suggesting that clearance of chylomicron
284 remnants is not improved nor impaired as a consequence of the reduced lipemia in
285 the earlier postprandial phase. This agrees with our earlier study showing that total
286 plasma apolipoprotein B48 concentrations did not differ over an 8 h postprandial
287 period following 50 g IPO and NPO in younger men and women [10].

288 This observed difference in the postprandial lipemic response might be due to
289 differences in the rate of intestinal absorption of the test fats. Palmitic acid profiles in
290 the plasma TAG fraction and the proportions of palmitic acid in isolated chylomicron
291 TAG fractions were similar following NPO and IPO as observed in our previous
292 studies [7, 10], suggesting that both fats were absorbed at similar rates. However

this is inconclusive unless tracer-labelled palmitic acid is ingested, as total plasma TAG fraction palmitic acid also reflects VLDL palmitic acid and/ or chylomicron palmitic acid released from stored TAG within the enterocytes derived from a previous meal. Evidence suggests that palmitic acid in the *sn*-2 position is unlikely to delay or reduce absorption in adults compared with TAG containing palmitic acid in the *sn*-1,3 positions [9]. It is therefore likely that the reduced lipemia observed following interesterification of palmitic acid-rich fats is due to changes in the physical characteristics of the fats, whereby the process of interesterification generates TAG species such as tri-palmitin and di-saturated TAG, which have melting points higher than body temperature. Indeed, in the current study the IPO contained a greater proportion of solid fat at 37 °C compared to NPO which was liquid at 37 °C and it is likely that the different melting points of the two test fats influenced the rate of digestion and absorption in the gut [5].

A slower rate of absorption may have initially lowered the lipemic response but ultimately resulted in increased plasma TAG concentrations in the very late postprandial period, beyond the 6 h period monitored in the current study; this was observed by Sanders et al (2011), where plasma TAG concentrations remained at about 28% above fasting concentrations 8 h following IPO, compared to only about 10% above fasting concentrations 8 h following NPO [10]. The duration of postprandial measurements is a limitation of the present study design as the effects on lipemia/TAG-rich lipoprotein composition beyond 6 h, and following subsequent meals, would further elucidate any differences as a result of interesterification of palm olein. Future studies should investigate compositional changes in the lipoprotein fractions at 8 and 10 h postprandially to achieve a better understanding of the influence of prolonged lipemia on the atherogenic potential of lipoproteins.

In summary, these compositional data from the lipoprotein fractions suggest that remnant triacylglycerol-rich lipoproteins are not differentially affected by palm oil interesterification up to 6 h, and imply that there is unlikely to be a greater risk of atherosclerosis when habitually consuming interesterified palm olein compared to native palm olein. However, further detailed investigations into lipoprotein fractions including the later postprandial phase (up to 8 or 10 h) and during chronic dietary interventions are required to confirm that compositional changes do not occur due to slower absorption and prolonged lipemia.

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Fig. 1. CONSORT flow diagram. CONSORT, Consolidated Standards of Reporting Trials.

Fig. 2 A Plasma and chylomicron fraction triacylglycerol (TAG) concentrations following 75 g native palm olein (closed symbols) and interesterified palm olein (open symbols). Mean with 95% CI, $n = 11$. Plasma TAG data were analysed by ANOVA on changes from fasting values: 1-6 h meal effect $P = 0.067$, meal x time interaction $P = 0.469$, and time effect $P < 0.0005$; 1-4 h meal effect $P = 0.020$, meal x time interaction $P = 0.316$, and time effect $P < 0.0005$. Chylomicron fraction data were analysed by ANOVA on raw values: 4-6 h meal effect $P = 0.055$, meal x time interaction $P = 0.147$, and time effect $P = 0.037$. **B** Paired T-test of the iAUC 1-6 h for plasma TAG showed a non-significant difference between meals ($P = 0.090$) but paired T-test of the iAUC 1-4 h for plasma TAG showed a significant reduction following IPO compared to NPO ($P = 0.024$); error bars are 95% CI. **C** Plasma non-esterified fatty acids (NEFA) concentrations following 75 g native palm olein (closed symbols) and interesterified palm olein (open symbols). Mean with 95% CI, $n = 10$. Data were analyzed by ANOVA on changes from fasting values: meal effect $P = 0.928$, meal x time interaction $P = 0.572$ and time effect $P < 0.0005$. **D** Plasma cholesterol concentrations following 75 g native palm olein (closed symbols) and interesterified palm olein (open symbols). Mean with 95% CI, $n = 11$. Data were analyzed by ANOVA on changes from fasting values: meal effect $P = 0.613$, meal x time interaction $P = 0.102$ and time effect $P = 0.001$.

Table 1. Proportions of fatty acids in the total and *sn*-2 positions of the experimental test fats.

Fatty acids (mol %)		Native palm olein	Interesterified palm olein
12:0	Total	0.3	0.4
	<i>sn</i> -2	0.0	0.0
14:0	Total	1.1	1.2
	<i>sn</i> -2	0.6	1.3
16:0	Total	41.7	42.9
	<i>sn</i> -2	9.8	45.9
18:0	Total	4.2	4.5
	<i>sn</i> -2	1.4	4.8
16:1	Total	0.2	0.2
	<i>sn</i> -2	0.3	0.3
18:1n-9	Total	40.6	40.1
	<i>sn</i> -2	68.4	38.9
18:1n-7	Total	0.7	0.7
	<i>sn</i> -2	0.0	0.0
18:2n-6	Total	10.5	9.7
	<i>sn</i> -2	19.6	8.7
18:3n-3	Total	0.2	0.1
	<i>sn</i> -2	0.0	0.1
18:3n-6	Total	0.1	0.1
	<i>sn</i> -2	0.0	0.0
20:3n-6	Total	0.1	0.1
	<i>sn</i> -2	0.0	0.0
20:4n-6	Total	0.2	0.1
	<i>sn</i> -2	0.0	0.0

Table 2. Characteristics of healthy male subjects who completed the study (n=11)^a.

Variable	
Age, y	50 ± 7
Body Mass Index, kg/m^2	27.6 ± 3.1
Systolic blood pressure, <i>mmHg</i>	122.2 ± 14.0
Diastolic blood pressure, <i>mmHg</i>	80.1 ± 10.2
Waist circumference, <i>cm</i>	98.6 ± 9.9
Body Fat, %	26.3 ± 5.6
Fasting plasma glucose, <i>mmol/L</i>	5.6 ± 0.3
Fasting plasma triacylglycerol, <i>mmol/L</i>	1.8 ± 0.3
Fasting plasma total cholesterol, <i>mmol/L</i>	6.3 ± 0.5
Fasting plasma LDL cholesterol, <i>mmol/L</i>	4.1 ± 0.5
Fasting plasma HDL cholesterol, <i>mmol/L</i>	1.4 ± 0.3

^aValues are means ± SD.

Table 3 Triacylglycerol and cholesterol composition of the chylomicron and IDL plasma lipoprotein fractions 4-6 h following consumption of meals containing 75 g native palm olein versus interesterified palm olein.

	Native palm olein			Interesterified palm olein			P value
	4h	5h	6h	4h	5h	6h	(meal effect)
TAG (mmol/L)							
Chylomicron fraction	1.43	1.25	1.04	0.97	1.03	0.85	0.055
	(1.08-1.79)	(0.95-1.55)	(0.84-1.24)	(0.70-1.24)	(0.78-1.28)	(0.63-1.06)	
IDL fraction	1.89	1.70	1.70	1.87	1.84	1.76	0.693
	(1.33-2.44)	(1.40-2.00)	(1.38-2.01)	(1.54-2.21)	(1.63-2.04)	(1.55-1.98)	
Cholesterol (mmol/L)							
Chylomicron fraction	0.36	0.37	0.32	0.32	0.35	0.29	0.498
	(0.26-0.46)	(0.30-0.45)	(0.24-0.39)	(0.22-0.42)	(0.25-0.44)	(0.18-0.40)	
IDL fraction	1.26	1.26	1.26	1.57	1.66	1.47	0.229
	(0.85-1.67)	(0.88-1.64)	(0.85-1.67)	(1.12-2.03)	(1.11-2.22)	(0.99-1.95)	
TAG/Cholesterol ratio							
Chylomicron fraction	4.03	3.36	3.41	3.22	3.11	3.43	0.268
	(3.55-4.51)	(2.72-4.00)	(2.88-3.95)	(2.75-3.69)	(2.71-3.51)	(2.53-4.33)	

IDL fraction	1.66 (1.41-1.92)	1.60 (1.44-1.76)	1.81 (1.69-1.92)	1.40 (1.35-1.44)	1.30 (1.13-1.48)	1.40 (1.32-1.49)	0.088
Apo B48 in IDL fraction (ug/ml) ^{ab}	15.02 (2.62-27.42)	15.42 (7.00-23.84)	17.89 (9.72-26.05)	17.94 (4.81-31.07)	19.10 (7.08-31.13)	17.20 (3.48-30.93)	0.405

Values are means (95% confidence interval). ^a N=8 due to missing sample; all other data n=11. ^b Repeated ANOVA carried out on natural log transformed data.

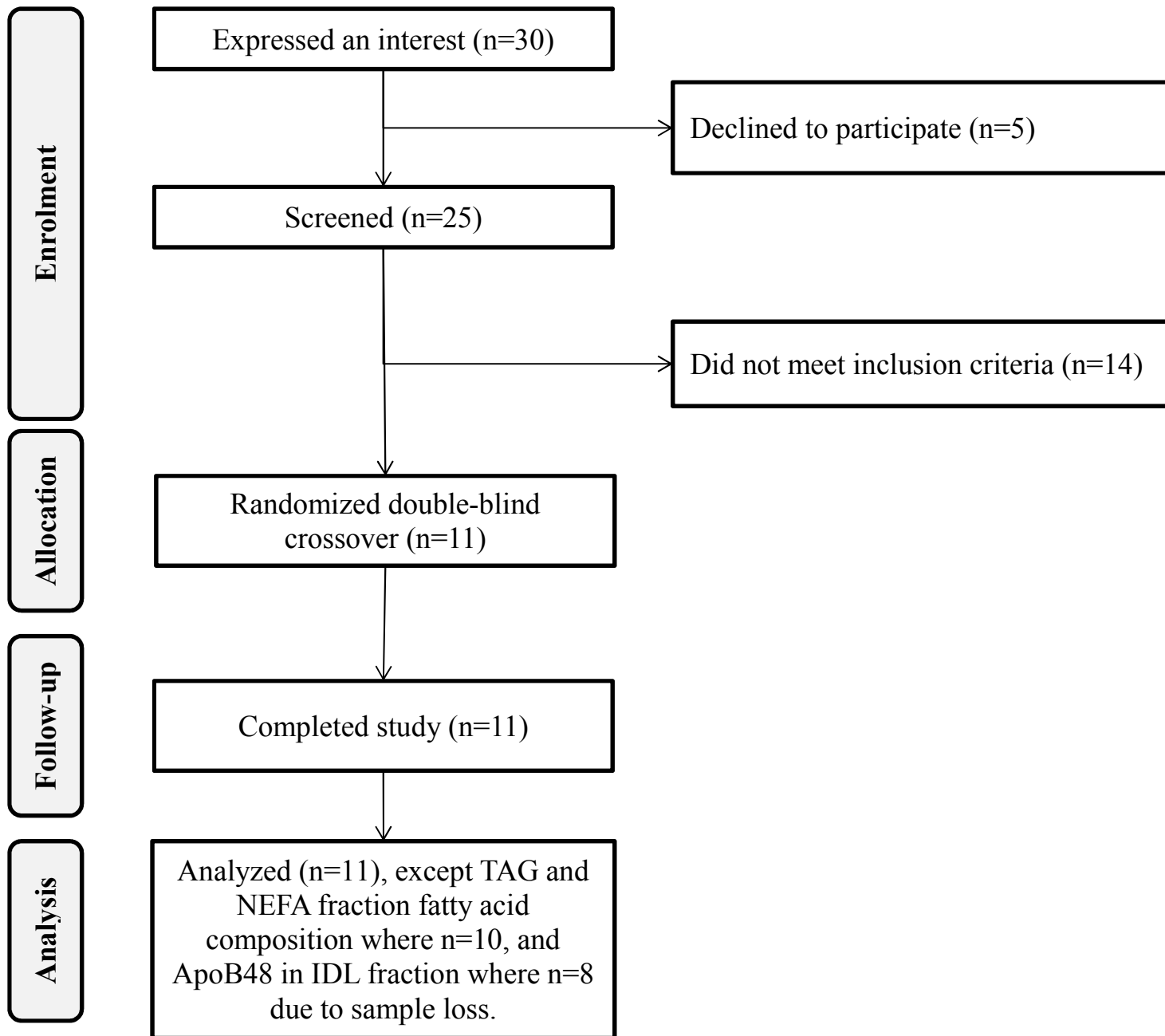
Table 4. Proportions of fatty acids in plasma triacylglycerol and non-esterified fatty acid fractions during the 6 h postprandial period following consumption of meals containing 75 g native palm olein versus interesterified palm olein.

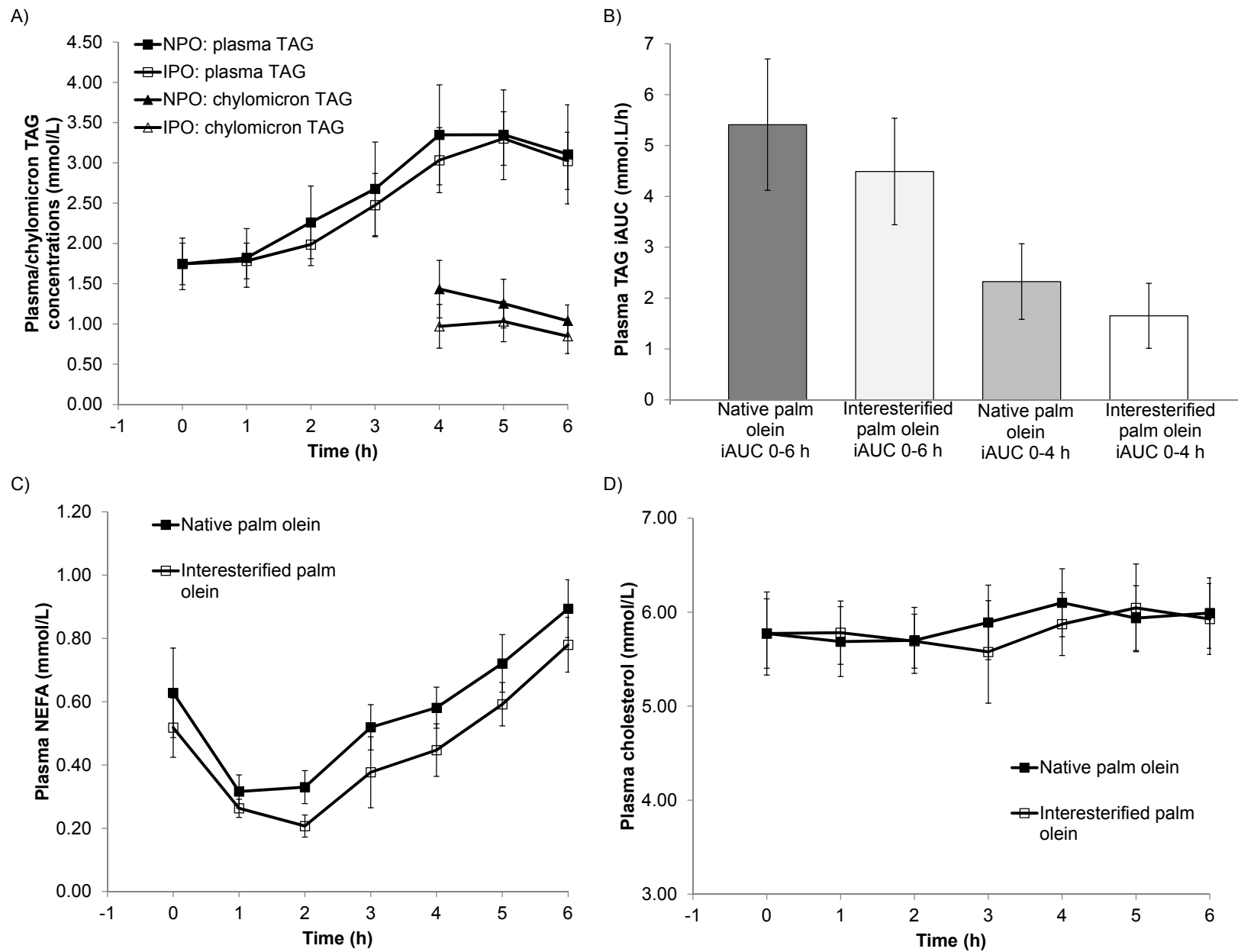
Fatty acid (mol%)	TAG fraction		NEFA fraction	
	Native palm olein	Interesterified palm olein	Native palm olein	Interesterified palm olein
16:0				
Baseline	29.2 ± 3.5	29.1 ± 2.6	35.4 ± 10.4	35.8 ± 10.2
1 h	31.4 ± 5.2	29.0 ± 2.3	37.8 ± 13.2	40.3 ± 13.4
2 h	31.7 ± 4.3	32.8 ± 6.5	41.0 ± 14.9	39.7 ± 10.5
3 h	33.3 ± 2.6	34.4 ± 9.4	40.1 ± 12.5	42.9 ± 13.5
4 h	36.6 ± 8.7	34.7 ± 4.7	40.5 ± 12.4	42.1 ± 12.1
5 h	33.1 ± 3.0	33.6 ± 4.7	41.9 ± 13.7	41.7 ± 12.5
6 h	33.3 ± 4.1	36.4 ± 11.4	40.8 ± 13.1	40.2 ± 14.2
18:0				
Baseline	3.3 ± 0.8	3.1 ± 0.7	10.5 ± 3.4	9.5 ± 2.9
1 h	3.5 ± 0.8	3.2 ± 0.9	9.7 ± 3.8	9.5 ± 3.1
2 h	4.1 ± 1.0	3.6 ± 1.3	8.8 ± 3.5	9.1 ± 3.7
3 h	3.6 ± 0.4	3.8 ± 1.4	8.2 ± 3.0	8.4 ± 2.8
4 h	3.7 ± 0.3	3.4 ± 1.2	8.2 ± 3.0	7.6 ± 2.3
5 h	4.1 ± 1.4	3.7 ± 0.5	8.4 ± 2.1	8.1 ± 2.6
6 h	3.3 ± 0.6	4.0 ± 1.3	8.4 ± 2.0	8.8 ± 3.0
18:1 n -9				
Baseline	32.6 ± 4.3	30.3 ± 7.5	22.2 ± 8.7	20.3 ± 8.9

1 h	31.8 ± 8.5	31.8 ± 8.3	15.2 ± 8.5	16.3 ± 8.9
2 h	33.5 ± 3.3	31.5 ± 8.4	15.4 ± 10.0	15.9 ± 8.7
3 h	35.4 ± 2.7	30.8 ± 8.5	18.4 ± 9.2	16.8 ± 10.8
4 h	36.2 ± 3.5	35.7 ± 2.7	18.8 ± 9.4	19.9 ± 9.3
5 h	36.2 ± 3.0	35.0 ± 3.2	20.7 ± 9.7	20.6 ± 9.7
6 h	33.8 ± 8.0	35.2 ± 1.8	21.4 ± 9.0	17.6 ± 9.2
18:2<i>n</i>-6				
Baseline	11.4 ± 1.4	10.8 ± 2.3	7.0 ± 3.5	6.4 ± 2.6
1 h	12.0 ± 1.8	11.7 ± 2.2	5.8 ± 4.6	5.4 ± 2.6
2 h	10.7 ± 2.5	10.0 ± 2.7	6.5 ± 5.9	5.9 ± 3.2
3 h	11.2 ± 1.4	9.3 ± 3.0	6.8 ± 3.0	5.3 ± 3.2
4 h	10.8 ± 1.2	10.2 ± 1.1	6.6 ± 5.3	6.2 ± 3.0
5 h	10.8 ± 1.2	10.1 ± 1.8	6.0 ± 2.7	6.0 ± 2.8
6 h	11.1 ± 1.7	10.1 ± 1.3	6.8 ± 2.8	5.6 ± 3.3

Data are means ±SD. N=10, except for TAG 18:1*n*-9 and TAG 18:2*n*-6 where N=9.

There were no meal effects or meal x time interactions for the compositions (mol %) of 16:0 (palmitic acid), 18:0 (stearic acid), 18:1*n*-9 (oleic acid), and 18:2*n*-6 (linoleic acid) in plasma TAG and NEFA fractions during the postprandial period. Time effects: there were significant increases in TAG 16:0, $P < 0.005$; NEFA 16:0, $P < 0.0005$; TAG 18:1*n*-9, $P < 0.005$; and significant decreases in NEFA 18:0, $P < 0.005$; TAG 18:2*n*-6, $P < 0.05$; and an initial sharp decrease 1-2 h in NEFA 18:1*n*-9, followed by a return to baseline levels 3-6 h, $P < 0.005$. There were no time effects for plasma TAG fraction 18:0 mol % composition, nor plasma NEFA fraction 18:2*n*-6.





Supplementary Figure 1.

Vascular function and blood pressure following 75 g native palm olein (closed symbols) and interesterified palm olein (open symbols). Mean with 95% CI, n = 11. Data were analyzed by ANOVA on changes from fasting values. **A** Digital volume pulse stiffness index (DVP-SI): meal effect $P = 0.447$, meal x time interaction $P = 0.342$, and time effect $P = 0.711$. **B** Digital volume pulse reflection index (DVP-RI): meal effect $P = 0.872$, meal x time interaction $P = 0.474$, and time effect $P = 0.002$. **C** Systolic blood pressure (SBP): meal effect $P = 0.433$, meal x time interaction $P = 0.015$ and time effect $P < 0.0005$. **D** Diastolic blood pressure (DBP): meal effect $P = 0.496$, meal x time interaction $P = 0.706$ and time effect $P < 0.0005$.

